

Observations on the reproductive biology of the Bull ray, *Pteromylaeus bovinus* (E. GEOFFROY SAINT-HILAIRE, 1817) (Chondrichthyes: Myliobatidae) from the coast of Senegal (Eastern tropical Atlantic)

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The bull ray, *Pteromylaeus bovinus* (E. GEOFFROY SAINT-HILAIRE, 1817) is commonly caught off the coast of Senegal. Adult males and females were over 820 mm and 900 mm disk-width (DWd) respectively, with the largest male and the largest female recorded being 1150 mm and 1480 mm DWd respectively. They weighed 29.8 kg and 47.9 kg respectively. The females were significantly heavier than the males. The DWd of eight fully developed fetuses ranged from 250 to 270 mm DWd (mean: 258.70 ± 8.33), their total length (TL) from 530 to 560 mm (mean: 541.27 ± 11.20) and their weight from 310 to 345 g (mean: 326.25 ± 13.29). The smallest free-living specimen was a female; its DWd was 355 mm, its TL 585 mm and it weighed 460 g. Diameter of ripe oocytes ranged from 22 to 26 mm (mean: 23.6 ± 0.14); they weighed from 4.1 to 6.3 g (mean: 5.25 ± 0.61). Gestation lasted between 5 and 6 months. It appeared a block of oocytes at the beginning of gestation and there was an inability to ovulate soon after parturition. Vitellogenesis recommenced when the embryos were practically at the end of their development. The reproductive cycle lasted one year at least. A calculated chemical balance of development based on mean dry weights of the fully developed fetuses and the ripe oocytes is 31.12. This high value shows that *P. bovinus* is a pure matrotrophic species. Counts of ripe oocytes, eggs, embryos and fully developed fetuses showed that ovarian fecundity is higher than uterine fecundity. The former ranged from 6 to 8, the latter from 3 to 4. There were no relationship between size and both categories of fecundity. In the sample, both adult males and females were more abundant than the juvenile ones. Among the adults, males were significantly more numerous than females.

Key words: Chondrichthyes, Myliobatidae, *Pteromylaeus bovinus*, reproductive biology, Senegal, Eastern tropical Atlantic

INTRODUCTION

Preliminary data on the reproductive biology of the bull ray, *Pteromylaeus bovinus* (G. SAINT-HILAIRE, 1817) from the coast of

Senegal were reported by CAPAPÉ *et al.* (1995). New records of *P. bovinus* allow these data to be expanded. This paper details size at sexual maturity, size at birth, reproductive cycle and

fecundity. They were compared with data provided by CAPAPÉ and QUIGNARD (1975) from *P. bovinus* caught in the Tunisian waters.

MATERIAL AND METHODS

The bull rays were caught off the coast of Senegal from 1994 to 2000. They were generally captured by demersal gill nets and rarely by trawling, in the shallow coastal waters at a depth of 80 m maximum, on sandy and/or muddy bottoms, rarely among macroalgae. They were landed at different fishing-sites along the coast of Senegal, but most of our observations were made at Ouakam (Fig. 1).

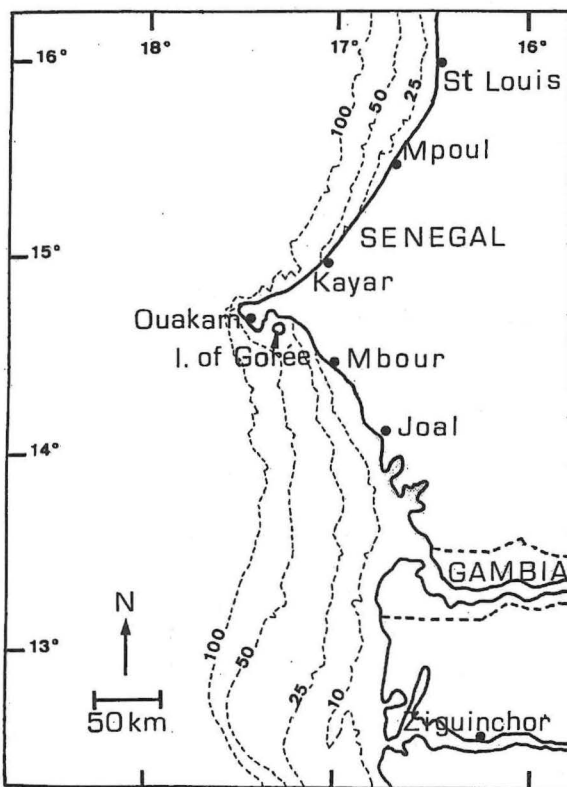


Fig. 1. Map of the coast of Senegal (redrawn by KÉBÉ and LE RESTE, 1993) pointing out the landing sites of *Pteromylaeus bovinus*)

A total of 80 specimens were examined, including 44 males and 36 females. The monthly collection, the sex and the condition of the observed specimens are summarized in Table 1. In addition, 14 embryos and eight fetuses were

studied. Embryos still had an umbilical stalk and a yolk stalk. These organs were completely resorbed in fully developed fetuses. A scar marked the place of the umbilical stalk. The yolk stalk was reabsorbed in an internal vitelline vesicle.

The specimens were measured to the nearest millimeter for disk-width (*DWd*) following CLARK (1926) for skates. They were weighed to the nearest gram. Measurements also included clasper length (*CL*, mm) from the forward rim of the pelvic girdle to the tip of claspers according to COLLENOT (1969), the diameter of ripe and developing oocytes. All the oocytes, the embryos and the fully developed fetuses were weighed to the nearest decigram. They were removed from the ovaries and the uteri and then measured and weighed. Fetuses were sexed and the embryos, whenever possible.

The onset of sexual maturity was determined by the relationship between *CL* and *DWd*. The linear regression was expressed in decimal logarithmic coordinates. Correlation was assessed by least-squares regression. BASS *et al.* (1975) noted that claspers of juveniles are short and flexible, while males are adult when claspers are rigid, elongated and calcified. These characteristics were verified for *P. bovinus* in order to avoid underestimation of size at maturity.

Size of females at sexual maturity was determined from the condition of ovaries and the morphology of the reproductive tract. Three categories were distinguished in females as well as in males.

To investigate the embryonic development and the role of the mother during gestation, a chemical balance of development (*CBD*) was considered. *CBD* is based on the mean dry weight of fertilized eggs and fully developed fetuses. *CBD* can be computed as the mean dry weight of fully developed fetuses divided by the mean dry weight of fertilized eggs. Water content of 50 % ova and 75 % in fully developed fetuses can be taken as standard values, based on chemical analyses of the small spotted catshark, *Scyliorhinus canicula* by MELLINGER and WRISEZ (1989). *CBD* is a tentative estimate.

Table 1. Monthly collection of *Pteromylaeus bovinus* caught off the coast of Senegal

	Months												Total
	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	
Males													
Juveniles	3	2	-	-	2	1	1	-	-	-	-	1	10
Subadults	1	-	1	2	2	1	1	-	-	3	-	1	12
Adults	5	-	-	4	6	6	-	1	-	-	-	-	22
Total	9	2	1	6	10	8	2	1	-	3	-	2	44
Females													
Juveniles	2	-	1	-	2	1	1	2	-	-	-	2	11
Subadults	-	-	1	-	1	2	3	-	-	-	-	-	7
Adults	-	-	-	2	4	4	3	2	2	1	-	-	18
Total	2	-	2	2	7	7	7	4	2	1	-	-	36
General total	11	2	3	8	17	14	9	6	2	4	0	4	80

Tests for significance ($p < 0.05$) were performed by using analysis of variance, STUDENT t -test, χ^2 test. For relationship size vs. weight-disk-width, fitted lines were compared by ANCOVA.

RESULTS

Onset of sexual maturity

Males

The CL vs. DWd relationship shows two inflexions corresponding to three sexual maturity stages in males (Fig. 2).

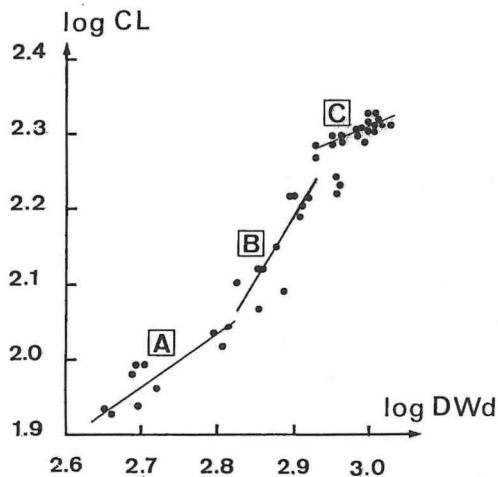


Fig. 2. Clasper length (CL) vs disk-width (DWd) expressed in decimal logarithmic coordinates. A. Juveniles. B. Subadults. C. Adults

The first stage included the juveniles. Their DWd ranged from 450 to 650 mm. The relationship is $\log [CL] = 0.586 \log [DWd] + 0.382$; $r = 0.907$; $n = 10$. The second stage concerned the subadults. DWd is between 720 and 850 mm and the relationship becomes: $\log [CL] = 2.514 \log [DWd] - 5.112$; $r = 0.900$; $n = 12$. The third stage included the adults. Their DWd ranged from 820 to 1150 mm, with $\log [CL] = 0.472 \log [DWd] + 0.885$; $r = 0.907$; $n = 22$.

According to these relationships, the claspers grew the fastest during the second stage. Juveniles and subadults had short, uncalcified and flexible claspers. Those of adults were elongated, calcified, rigid and functional. The third relation shows that they grew allometrically throughout life. All the males having more than 820 mm DWd were adult. The largest male, 1150 mm DWd , weighed 29.8 kg.

Concomitantly, during their growth, the males developed pre-orbital tubercles (Fig. 3 a,b,c, d).

CAPAPÉ and QUIGNARD (1975) described these features for the first time in *P. bovinus* from the Tunisian coasts. During their development, three stages were considered which occurred in male bull rays from Senegal. During stage I, the juveniles had the anterior edge of the orbits entirely smooth (Fig. 3 b). Stage II occurred during the phases of maturation where

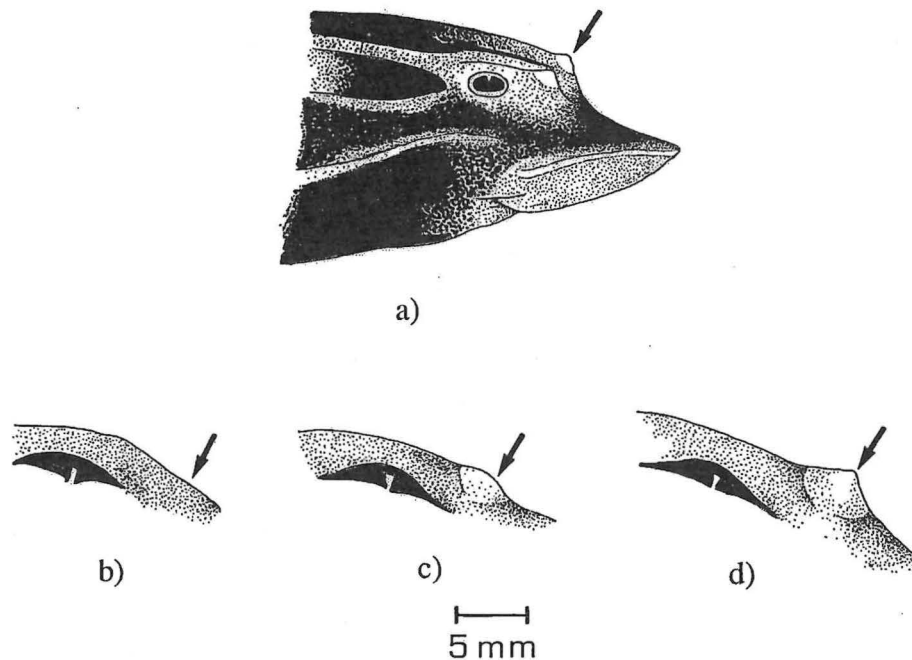


Fig.3. a) Head of *Pteromylaeus bovinus*, redrawn and adapted from CAPAPÉ and QUIGNARD (1975) showing the pre-orbital tubercles (arrow); b) Stage I. The anterior edge of the orbits is entirely smooth (arrow); c) Stage II. The orbital anterior edge shows a conspicuous protuberance (arrow); d) Stage III. The cone shaped pre-orbital tubercles (arrow) are well-developed

in subadults, the orbital anterior edge showed conspicuous protuberance (Fig. 3 c). In stage III, the adults exhibited cone shaped pre-orbital tubercles that were well developed (Fig. 3 d).

Females

As in males, three stages and three categories of females were considered. During the juvenile stage, the females had whitish ovaries with oocytes of microscopic size and inconspicuous nidamental glands. Eleven specimens were observed, their size ranged from 350 to 670 mm.

The subadult stage concerned seven females, which were between 720 and 880 mm *DWd*. They had translucent oocytes and a differentiated genital duct.

Eighteen adults were observed. Their size ranged from 900 to 1480 mm *DWd*. All these specimens showed a fully developed genital duct. The smallest female with ripe oocytes was 900 mm *DWd*, its weight reached 6.8 kg. The smallest gravid specimen was 900 mm *DWd*

and weighed 9.3 kg. The greatest adult female weighed 47.9 kg. It contained fully developed fetuses.

Reproductive cycle

The bull ray is an aplacental viviparous species. Both ovaries were functional; the right being more developed than the left one that is sometimes rudimentary. They produced oocyte clutches similar in size and weight. Only one of these clutches developed into ripe oocytes, the other degenerated. Twenty ripe oocytes were measured. Their diameter ranged from 22 to 26 mm (mean: 23.6 ± 0.14); they weighed from 4.1 to 6.3 g (mean: 5.25 ± 0.61).

Table 2 shows that the oocytes ended their full development in April and May. Two females with ripe oocytes entering the genital tract were recorded in May. Females with eggs in uteri were observed in May and June, with developing embryos in July and August and with fully developed fetuses in September and October.

Table 2. Reproductive cycle of female *Pteromylaeus bovinus*: Condition of ovaries and uteri during gestation

Number of females	Month of catch	Ovarian activity	Diameter of oocytes (mm)	Uterine content	Uterine content (DWt, mm)
1	Apr.	Vitellogenesis	20-21	-	-
1	Apr.	Vitellogenesis	22-26	-	-
2	May	Vitellogenesis	23-25	-	-
2	May	Resting	-	Eggs	-
4	Jun.	Resting	-	Eggs	-
2	Jul.	Resting	-	Embryos	28-32
1	Jul.	Resting	-	?	?
1	Aug.	Vitellogenesis	3-5	Embryos	190-200
1	Aug.	Vitellogenesis	10-12	Embryos	210-220
1	Sep.	Vitellogenesis	10-11	Fetuses	250-256
1	Sep.	Vitellogenesis	10-12	?	?
1	Oct.	Vitellogenesis	18-19	Fetuses	255-275

At the beginning of embryonic growth, the ovaries were in a resting phase. Vitellogenesis did not proceed in parallel with gestation. It recommenced when the embryos were just before 190-200 mm *DWd*, at the end of their development.

Two females, caught in July and in September, had their uterus empty and distended, with developed villi on internal wall. Presumably, they expelled their uterine content during capture and/or handling by fishermen. The second female exhibited a conspicuous vitellogenetic activity.

A complete egg-capsule was clearly observed. It contained from three to four eggs.

It was diaphanous, brownish and ended with a short filament on both sides.

Size at birth

Two females measuring 1360 and 1480 mm *DWd* and weighing 41.4 and 47.9 kg both contained four fully developed fetuses. The *DWd* of these eight fetuses, four males and four females, ranged from 250 to 270 mm (mean: 258.70 ± 8.33), their *TL* from 530 to 560 mm (mean: 541.27 ± 11.20) and they weighed from 310 to 345 g (mean: 326.25 ± 13.29).

The smallest free-living specimen was a female. It exhibited an unhealed scar on its ventral face. Its *DWd* was 355 mm, its *TL*, 585 mm and it weighed 460 g.

Weight-growth vs. disk-width relationships

The total weight vs. disk-width relationships were for males: $\log [TWg] = 3.328 \log [DWD] - 5.868$; $r = 0.978$ and for females $\log [TWg] = 3.408 \log [DWD] - 6.055$; $r = 0.989$; $n = 36$. They did not show significant differences in slope ($p = 0.53$) and showed significant differences in intercept ($p = 0.01$) between sexes (Fig. 4).

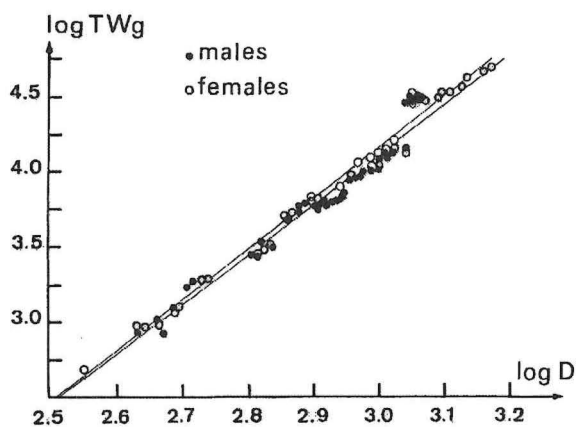


Fig. 4. Relationships total weight (TWg) vs. disk-width (Dwd) expressed in decimal logarithmic coordinates in male and in female *Pteromylaeus bovinus*

Chemical balance of development

Fresh weights of the 20 ripe largest oocytes ranged from 4.1 to 6.3 g (mean: 5.25 ± 0.61) and of 8 fully developed fetuses from 310 to 345 g (mean: 326.25 ± 13.29). CBD based on mean dry weights calculated for *P. bovinus* is 31.12.

Fecundity

Ovarian fecundity was based on the number of ripe oocytes ready for ovulation, counted in females from 900 to 1480 mm *Dwd*. This number ranged from six to eight. There is no relationship between size and ovarian fecundity.

Uterine fecundity based on the number of fertilized eggs, embryos and fully developed fetuses was counted in females between 960 and 1360 mm *Dwd*. The number of these different features ranged from three to four. As well as for ovarian fecundity, there is no relationship size vs. uterine fecundity.

Table 3 shows that among the embryos and the fully developed fetuses, numbers of males and females are practically the same. Among free-living specimens, it is also the case in juveniles and adults. On the opposite, adult males are significantly more numerous than the female ones.

Table 3. *Pteromylaeus bovinus* sex ratio for each category of specimens and for the total sample

Category		Males	Females	Males/Females
Uterine contents	Embryos	5	5	1: 1
	Fetuses	4	4	1: 1
	Total	9	9	1: 1
Free-living specimens	Juveniles	10	11	1: 1.1
	Subadults	12	7	1.71: 1
	Adults	22	18	1: 1.62
	Total	44	36	1: 1.35
General total		53	47	1.12: 1

DISCUSSION

P. bovinus is an Atlanto-Mediterranean species from warm temperate and tropical waters (CAPAPÉ and QUIGNARD, 1975). Off Senegal, *P. bovinus* is commonly caught according to our observations, especially adults of both sexes in spring and summer. This semi-pelagic species approach the coast for mating and the pregnant females, which find nursery areas.

Off Senegal, males matured at a smaller size than females. This characteristic explains why the latter reached a greater size and were heavier than the former. This phenomenon is a new instance of size sexual dimorphism and agrees with MELLINGER (1989) who stated that it is practically the rule in elasmobranchs except in some scyliorhinids as *S. canicula*.

Off Tunisian coasts, male and female *P. bovinus* were adult at practically the same disk-width (CAPAPÉ and QUIGNARD, 1975). It is also the case for maximal disk-widths. Generally, 1.50 m is considered as the maximum disk-width for *P. bovinus* by authors. No intraspecific differences in size occur among populations from various areas, presumably owing to the semi-pelagic way of life of *P. bovinus*.

On the other hand, size at birth is also the same whatever the area. However, RANZI (1932) recorded a fully developed fetus which was 280 mm *DWd* and weighed 470 g. The smallest free-living specimen observed in Senegal was 355 mm *DWd* and weighed 460 g.

The development of the pre-orbitary tubercles in three stages corroborated the previous description of CAPAPÉ and QUIGNARD (1975) for male bull rays from the Tunisian coasts. This morphological character was observed in all adult males from the western coast of Africa (FISCHER *et al.*, 1981).

The eggs are not heavy. Their weight ranged from 3 to 4 g in Tunisia and reached more than 7 g in specimens from Italy (RANZI, 1934). The ripe oocytes mean-weight for specimens from Senegal are 5.25 g.

Female *P. bovinus* were greater and heavier than male. It is generally the case in viviparous species, purely lecithotrophic as squatinids (CAPAPÉ *et al.*, 1990), squalids (GUALLART and VICENT, 2001), semi-lecithotrophic as torpedinids (MELLINGER, 1981; CAPAPÉ *et al.*, 2001), or purely matrotrophic as rhinopterids (HAMLETT *et al.*, 1985), dasyatids (CAPAPÉ, 1993) and gymnurids (CAPAPÉ *et al.*, 1992). The matrotrophy in *P. bovinus* following the definition of WOURMS *et al.* (1988) was confirmed by the computed *CBD* which revealed the highest values ever calculated in elasmobranchs, more than this reported by CAPAPÉ *et al.* (1992), 30.6 for the butterfly ray, *Gymnura altavela*, from the Tunisian coasts.

According to the data given in Table 2, females with fertilized eggs occurred in May and June, while females with embryos at different stages of their development occurred from June to August. On the other hand, females with fully developed fetuses were captured in September and in October. The gestation probably lasted between 5 and 6 months. In Tunisian waters, CAPAPÉ and QUIGNARD (1975) reported that it lasted between 5 and 8 months maximum.

From Italian waters, RANZI (1934) observed in June two females containing eggs *in utero* and referring to LO BIANCO (1909), he suggested that *P. bovinus* gestation lasted four months in this marine area.

Previously, CAPAPÉ and QUIGNARD (1975) reported that vitellogenesis started again at the end of the gestation in *P. bovinus* from Tunisian waters but they did not comment this phenomenon. Similar features occurred in specimens from the coast of Senegal. It suggests that vitellogenesis was partially delayed in females *P. bovinus* during gestation. The same phenomenon was described by CAPAPÉ *et al.* (1990) in *S. oculata* and *S. squatina* off the Tunisian coasts. This block of vitellogenesis was defined "semi-delayed vitellogenesis" by CAPAPÉ *et al.* (1990). These observations agree with COUSSEAU (1973), NATANSON and CAILLIET (1986) and SUNYE and VOOREN (1997). It seems to be the rule in squatinids.

The semi-delayed vitellogenesis is a mid-term feature with regard to the delayed vitellogenesis in torpedinids (MELLINGER, 1981), the *Centroscymnus* spp. (YANO and TANAKA, 1987, 1988) and probably the angular rough shark (CAPAPÉ *et al.*, 1999). Nevertheless, the vitellogenesis proceeds in parallel with gestation such as in *Squalus* spp. and other viviparous placental species (MELLINGER, 1989). However, in squatinids this block was canceled when embryos were at mid-term of their development and practically at the term in *P. bovinus*. So, it remains difficult to estimate the reproductive cycle length of *P. bovinus* regarding to this of vitellogenetic activity. The observations summarized in Table 2 suggest that reproductive cycle lasted one year at least for *P. bovinus* from the coast of Senegal.

Counts of ripe oocytes, eggs, embryos and fully developed fetuses showed that ovarian fecundity is significantly higher than uterine fecundity. This suggests that ripe oocytes

entered into atresy and were not ovulated. On the other hand, the authors retain probable that some gravid females aborted during capture and landing. CAPAPÉ and QUIGNARD (1975) reported similar observations on female *P. bovinus* from the Tunisian coasts.

The sex ratio did not show differences between numbers males and females among uterine content and free-living specimens, except among adults where males were significantly more abundant than males. This is probably due to segregation of sexes during reproductive cycle rather than sex mortality.

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**Biološka zapažanja o reprodukciji raže, *Pteromylaeus bovinus*
(E. GEOFFROY SAINT-HILARIE, 1817)
(Chondrichthyes: Myliobatidae)
u obalnim vodama Senegala (istočni dio tropskog Atlantika)**

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SAŽETAK

Raža, *Pteromylaeus bovinus* (E.GEOFFROY SAINT-HILARIE, 1817) je vrsta koja se često lovi u sene-galskim priobalnim vodama. Odrasli mužjaci i ženke imaju širinu diska preko 820 mm za ženke i do 900 mm za mužjake. Najveći mužjak je dug 1150 mm, a ženka 1480 mm, a teški su 29.8 kg (mužjak) i 47.9 kg (ženka). Duljina diska osam potpuno razvijenih fetusa se kretala od 250 do 270 mm, dok je srednja vrijednost *DWd* iznosila 258.70 ± 8.33 ; ukupna duljina se kretala od 530 do 560 mm (srednja vrijednost: 541.27 ± 11.20), a njihova težina je 310 do 345 g (srednja vrijednost: 326.25 ± 13.29). Najmanji živi primjerak je bila ženka s duljinom diska 355 mm, ukupne duljine 585 mm i težine 460 g. Promjer zrelih oocita se kretao između 22 i 26 mm (srednja vrijednost: 23.6 ± 0.14), dok je težina varirala od 4.1 do 6.3 g (srednja vrijednost: 5.25 ± 0.61). Gravidnost je trajala između 5 - 6 mjeseci. Na početku trudnoće se pojavila blokada oocita, a također je postojala i nemogućnost ovulacije ubrzo nakon poroda. Vitelogeneza je ponovno započela nakon što su embriji bili na samom kraju svog razvoja. Vrijeme reprodukcije je trajalo najmanje godinu dana. Izračun kemijske ravnoteže razvoja je načinjen prema srednjoj suhoj težini potpuno razvijenih fetusa i zrelih oocita, te iznosi 31.12. Ovako velika vrijednost pokazuje da je *P. bovinus* čisto matrotrofna vrsta. Broj zrelih oocita, jaja, embrija i potpuno razvijenih fetusa pokazao je da je ovarijalni fekunditet veći od uterinog. Prethodni je iznosio 6-8, a potonji 3-4. Ne postoji povezanost između veličina i dviju kategorija plodnosti. U uzorku su odrasli mužjaci i ženke brojniji od juvenilnih primjeraka, s tim da su odrasli mužjaci brojniji od odraslih ženki.

Ključne riječi: Chondrichthyes, Miliobatidae, *Pteromylaeus bovinus*, biologija reprodukcije, Senegal, istočni tropski Atlantik